

# Bug pheromones (Hemiptera, Heteroptera) and tachinid fly host-finding<sup>1,2</sup>

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**Abstract:** Data and observations on wild tachinid flies that were attracted to traps baited with known or suspected pheromones for the following stink bugs (Hemiptera: Heteroptera: Pentatomidae) are reported: *Podisus maculiventris* (SAY), *Euschistus tristigmus* (SAY), *Thyanta custator accerra* MCATEE, *Acrosternum hilare* (SAY), and *Halyomorpha halys* (STÄL). *Halyomorpha halys*, called the brown marmorated stink bug, is a newly invasive species in the eastern U. S., while the other stink bugs listed are native North American species. The following known tachinid parasitoids of stink bugs were captured: *Euclytia flava* (TOWNSEND) (Phasiinae), *Gymnosoma par* (WALKER) (Phasiinae), *Euthera tentatrix* LOEW (Dexiinae), *Hemyda aurata* ROBINEAU-DESVOIDY (Phasiinae), *Cylindromyia fumipennis* (BIGOT) (Phasiinae), and *Trichopoda pennipes* (F.) (Phasiinae). Tachinids in the subfamily Phasiinae commonly exploit pheromones to guide their search for potential hosts. The findings of the current study bolster this conclusion, and provide clues to pheromones and host/parasitoid relationships yet to be discovered.

**Key words:** Biological control, electrophysiology, host selection, kairomone, stink bug.

## Introduction

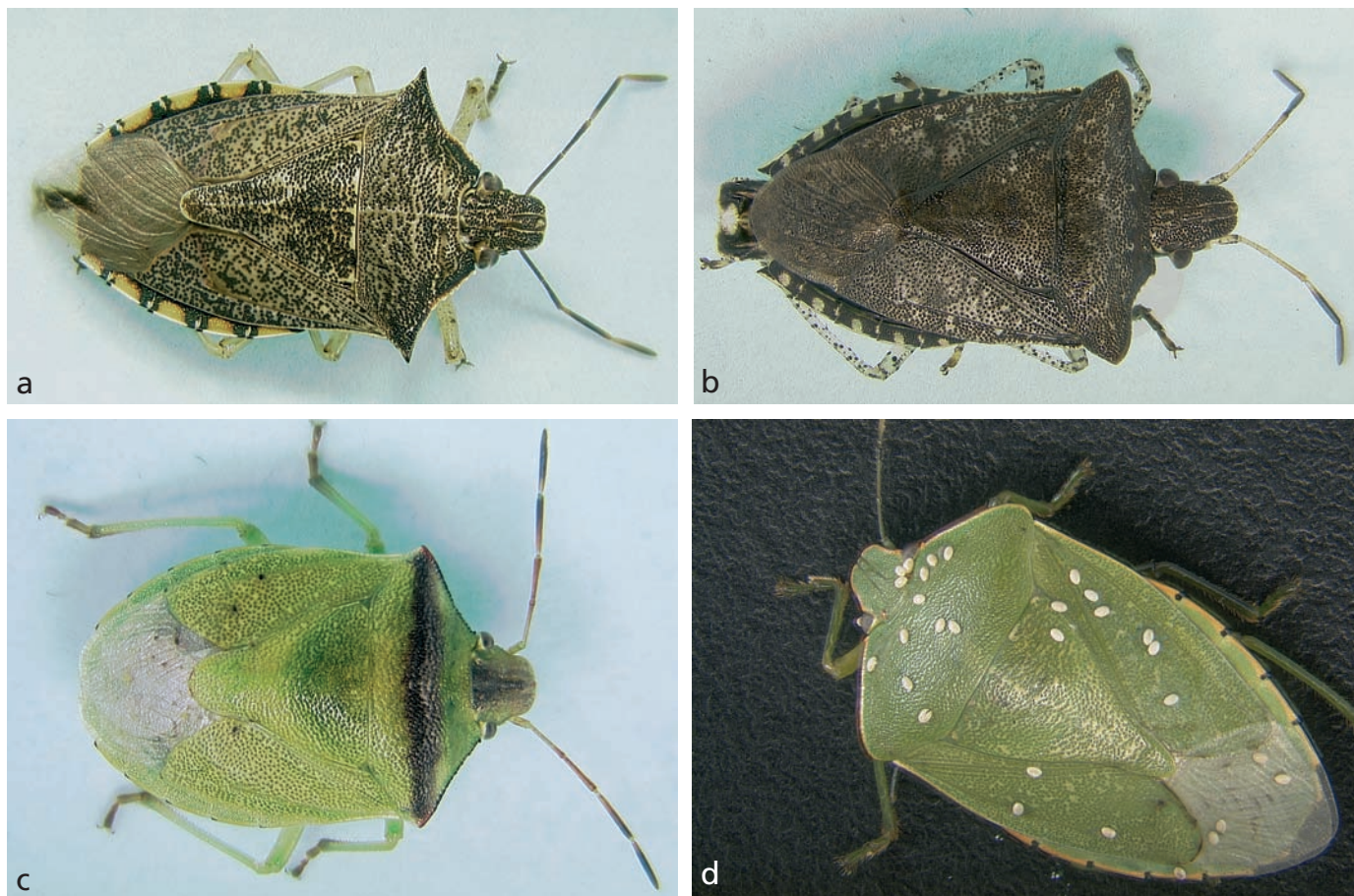
There are about 10,000 described species of tachinid flies worldwide – possibly only half the actual number of existing species – making this group one of, if not the most speciose family of the Diptera (STIREMAN III et al. 2006). All tachinid larvae are obligate endoparasites of arthropods, second only in importance for biological control to parasitic Hymenoptera. Members of the four recognized tachinid subfamilies (Exoristinae, Dexiinae, Tachininae, and Phasiinae) parasitize species from ten insect orders, plus some spiders, scorpions and centipedes (STIREMAN III et al. 2006). The Exoristinae parasitize mainly caterpillars and other exposed larvae, and this may be the ancestral lineage of Tachinidae. The most host-restricted tachinid subfamily, the Phasiinae, exclusively parasitize Heteroptera, especially adults, a specialization thought to have resulted from an early host shift from lepidopteran larvae (STIREMAN III et al. 2006).

Tachinids evidently evolved from a sarcophagid-like ancestor (STIREMAN III et al. 2006), and have yet to reach their evolutionary zenith (O'HARA 1985). Their larvae are relatively tolerant of toxins, a trait perhaps associated with their sarcophagous ancestry, and one that may have pre-adapted the group to exploit larvae (STIREMAN III et al. 2006) often protected by sequestration of secondary plant compounds (DUFFEY 1980). The evolutionary “jump” by phasiines to the Heteroptera may also have been a consequence, in part, of their tolerance for noxious compounds. Having accomplished the host shift to Heteroptera, phasiines have prolifically radiated in the comparatively enemy-free space provided by these chemically defended insects (JEFFRIES & LAWTON 1984; STIREMAN III et al. 2006).

Phasiines usually lay a large, so-called macrotypic egg on the cuticle of their host from which the larvae bore through the bottom of the egg into the haemocoel of the host (DUPUIS 1949). This type of oviposition is shared with their exoristine relatives,

<sup>1</sup> This paper is dedicated to Ernst Heiss on the occasion of his 70<sup>th</sup> birthday.

<sup>2</sup> Mention of commercial products does not constitute an endorsement by USDA.



**Fig. 1:** Known or suspected pheromone compounds of the following North American stink bugs (Pentatomidae) were tested for attraction and stimulation of native tachinids: A. *Podisus maculiventris* (SAY), B. *Euschistus tristigmus* (SAY), C. *Thyanta custator accerra* McATEE and, D. *Acrosternum hilare* (SAY) (with eggs of *Trichopoda pennipes* (F.); parasitism conditions in text).

although some members of both these subfamilies oviposit their eggs directly into the haemocoel of the host; for example, female *Leucostoma gravipes* (WULP) were often observed inserting their ovipositor into lygaeid bug hosts (ALDRICH et al. 1999). Other types of tachinid oviposition include laying microtype eggs nearby a host that may eventually ingest them, and larvipositing on or near hosts (O'HARA 1985; STIREMAN III et al. 2006). Host searching and selection by tachinids that attack herbivorous larvae commonly involves chemical cues associated with feeding hosts, such as damaged leaf odors (e.g., MONDOR & ROLAND 1998; KAINOH et al. 1999) or odors from frass (e.g., MONDOR & ROLAND 1997), but also frequently involves vision (STIREMAN III 2002b; YAMAWAKI & KAINOH 2005). In some cases physical cues such as texture may actually be more important for host selection than are chemical cues (e.g. DIPPEL & HILKER 1998), and learning probably improves the ability of tachinids to locate hosts (MONTEITH 1963; STIREMAN III 2002a). Tachinids that larviposit or lay

macrotype eggs usually exhibit little or no superparasitism (STIREMAN III et al. 2006). In general, however, tachinids do not recognize that a potential host has previously been parasitized (but see: LOPEZ & FERRO 1995) so, although only one parasitoid can survive, instances of multiple eggs on a single host do occur in nature, especially in agricultural contexts where potential hosts are abundant (TODD & LEWIS 1976). Sometimes tachinids co-opt defensive secretions of their hosts by homing-in on these allomones to locate potential hosts (ZVEREVA & RANK 2004), and some phasiines are attracted to nymphal allomones despite the fact that they more frequently parasitize the conspecific adult stage (ALDRICH 1988a).

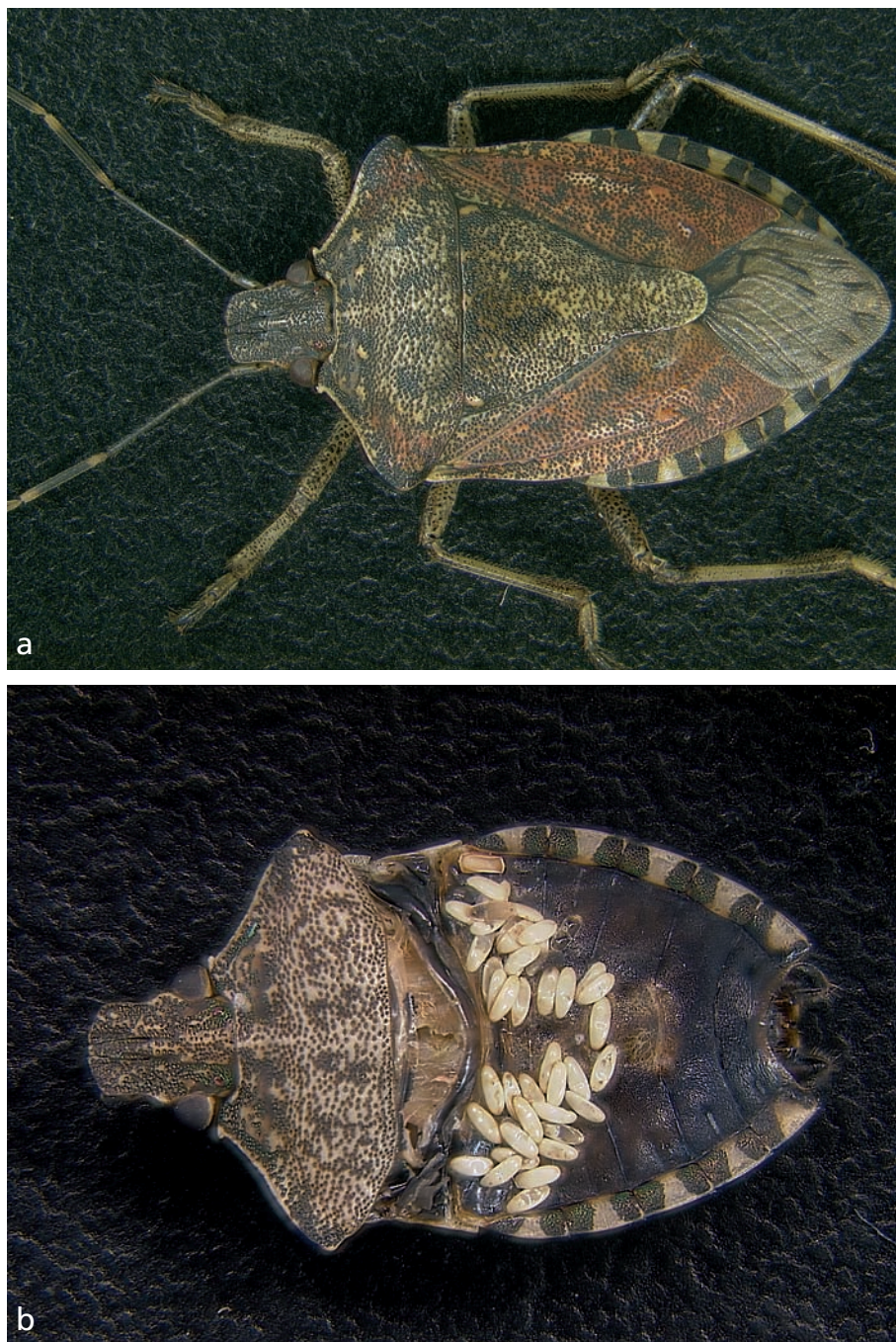
The mating songs of crickets and other orthopterans have been spectacularly subjugated by members of the tribe Ormiini (Tachininae) whose species have evolved tympanal ears (EDGEComb et al. 1995; ROBERT et al. 1996) so as to phonotactically find these hosts (CADE 1975; ZUK & KOLLURU 1998; ALLEN et al. 1999; STIREMAN III et al. 2006). This phenomenon is interesting be-



cause it entails the exploitation of sexual host signals (ZUK & KOLLURU 1998) resulting in trade-offs for calling males trying to attract a mate but simultaneously risking being found out by deadly parasitoids (STIREMAN III et al. 2006).

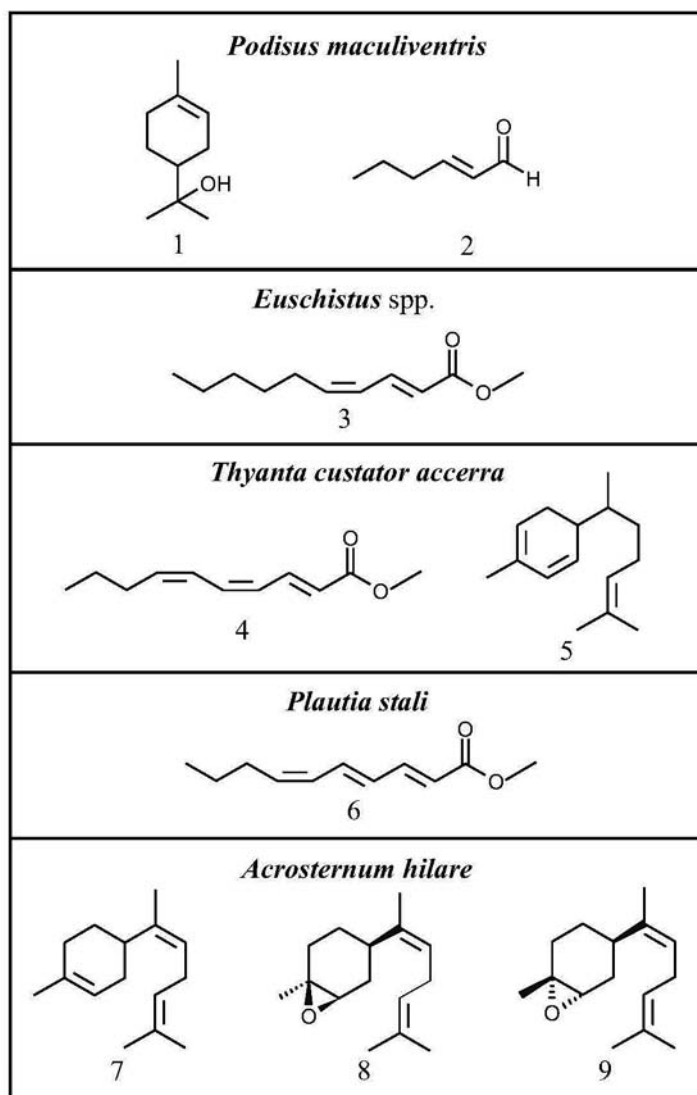
Host-finding by some, perhaps most, phasiine tachinids is analogous to that of the phonotactically orienting Ormiini, but instead of using acoustic signals many phasiines utilize the attractant pheromones of heteropterans to find these potential hosts (MITCHELL & MAU 1971; HARRIS & TODD 1980; ALDRICH et al. 1984; ALDRICH et al. 1987; ALDRICH et al. 1991; ALDRICH 1995b; ALDRICH et al. 1999; KRUPKE & BRUNNER 2003; JOHNSON et al. 2005). Indeed, the ability to capture large numbers of tachinids alive in traps baited with synthetic heteropteran pheromones has facilitated detailed studies of host location (ALDRICH & ZHANG 2002) and acceptance (ALDRICH 1995a) by *Euclytia flava* (TOWNSEND), a species that is extremely difficult to rear as are most tachinids. Gas chromatographic electroantennogram detector (GC-EAD) experiments using antennal preparations of wild *E. flava* revealed that pheromone strains of these flies exist (ALDRICH & ZHANG 2002), a phenomenon in keeping with earlier behavioral observations with another heteropteran tachinid parasitoid, *Trichopoda pennipes* F. (DIETRICK & VAN DEN BOSCH 1957; PICKETT et al. 1996). Moreover, GC-EAD experiments have shown that several phasiine parasitoids are at least as sensitive to the pheromones of their hosts as are the hosts themselves (ALDRICH & ZHANG 2002), a situation that has also been demonstrated for certain predacious clerid beetles that use bark beetle pheromones as prey-finding kairomones (HANSEN 1983).

In this paper we will report data and observations on wild tachinid flies that were attracted to traps baited with known or suspected pheromones for the following stink bugs (Pentatomidae): *Podisus maculiventris* (SAY) (ALDRICH et al. 1984) (Fig. 1A), *Euschistus tristigmus* (SAY) (ALDRICH et al. 1991) (Fig. 1B), *Thyanta custator accerra* MCATEE (MCBRIEN et al. 2002) (Fig. 1C), *Acrosternum hilare* (SAY) (ALDRICH et al. 1989; MCBRIEN et al. 2001) (Fig. 1D) and



*Halyomorpha halys* (STÅL) (Fig. 2A). *Halyomorpha halys*, called the brown marmorated stink bug, is a newly invasive species in the eastern U. S. (HOEBEKE & CARTER 2003), while the other stink bugs listed are native North American species. The pheromone for *H. halys* is unknown, but in Japan this bug was reportedly cross-attracted (TADA et al. 2001a, 2001b) to the pheromone of another stink bug, *Plautia stali* SCOTT (SUGIE et al. 1996). We are currently trying to identify the pheromone of *H. halys* and reinvestigating the pheromone systems of *Euschistus*,

**Fig. 2:** A. The Oriental species, *Halyomorpha halys* (STÅL), called the brown marmorated stink bug, is a newly invasive species in the eastern United States. B. Adults of *H. halys* exposed in the laboratory to field-collected *Gymnosoma par* (WALKER) (Tachinidae) were exclusively parasitized underneath the wings.



**Fig. 3:** Structures of known pentatomid pheromone molecules that were tested, and the species with which the compounds are associated: 1)  $\alpha$ -terpineol, 2) (E)-2-hexenal, 3) methyl (E,Z)-2,4-decadienoate, 4) methyl (E,Z,Z)-2,4,6-decatrienoate, 5) zingiberene, 6) methyl (E,E,Z)-2,4,6-decatrienoate, 7) (Z)- $\alpha$ -bisabolene, 8) (4S)-cis-(Z)- $\alpha$ -bisabolene epoxides and, 9) (4S)-trans-(Z)- $\alpha$ -bisabolene epoxides.

*Thyanta*, and other stink bugs economically important in the U. S.; these data will be presented elsewhere. Here we will focus on the use of these heteropteran pheromones as kairomones by various Tachinidae.

## Materials and Methods

### Chemical standards and treatments

Standards of the following compounds were purchased: (E)-2-hexenal, methyl (E,Z)-2,4-decadienoate (Bedoukian Research, Inc., Danbury, Connecticut);  $\alpha$ -terpineol (Fisher Scientific Company, Fair Lawn, New Jersey); and bisabolene (International Flavors & Fragrances, Union Beach, NJ). Methyl (E,Z,Z)-, (Z,E,Z)- and (E,E,Z)-2,4,6-decatrienoates were synthesized as previously described (Khrimian, 2005), as were (4S)-cis- and (4S)-trans-(Z)- $\alpha$ -bisabolene

epoxides ((Z)-(1R,2S,4S)-4-(1',5'-dimethyl 1',4'-hexadienyl)-1,2-epoxy-1-methylcyclohexane and (Z)-(1S,2R,4S)-4-(1',5'-dimethyl 1',4'-hexadienyl)-1,2-epoxy-1-methylcyclohexane, respectively) (CHEN et al. 2000). Zingiberene [5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene] was isolated from ginger essential oil using the procedure of MILLAR (1998). Structures of known pheromone molecules that were tested are shown in Fig. 3.

Lures for the initial experiment (Exp. 1) included treatments mimicking the natural pheromones of *P. maculiventris* (ALDRICH et al. 1984) and *T. custator accerra* (MCBRIEN et al. 2002), with five replicates per treatment prepared as follows. (E)-2-Hexenal and racemic  $\alpha$ -terpineol were mixed in a molar ratio of 1: 0.843. Batches of 40 ml of pheromone (17.6 ml hexenal, 21.4 ml  $\alpha$ -terpineol) were normally prepared. A 20% (v/wt) formulation of pheromone in plasticized polyvinyl chloride (PVC) was prepared by mixing 107 g of powdered PVC (Tenneco, Piscataway, NJ) with 53 g of dioctyl phthalate (Aldrich Chemicals) (FITZGERALD et al. 1973). The pheromone-PVC mixture was poured into test tubes (to the top), heated at 110 °C for 30 min, removed from the tubes, and stored in sealed vials in a freezer until use (treatment = "H+ $\alpha$ -T"). Approximately 1 g slices of PM were used to bait traps.

In Exp. 1, lures for treatments based on the *Thyanta* pheromone were prepared by placing ten rubber septa (West Pharmaceutical Services, Kearney, NE) in a 100 ml round-bottom one-neck flask, and covering the septa with an 8-ml hexane solution containing 44 mg of methyl (E,Z,Z)-2,4,6-decatrienoate plus 40 mg of zingiberene. The flask was rotated on a rotary evaporator (without applying a vacuum) for 1.5-2 h or until the liquid was almost completely absorbed into the septa. Septa were deployed in traps pinned inside ~7 cm-long pieces of standard PVC plumbing pipe (2.5 cm I.D.) to protect the lures from direct sunlight; this treatment is coded as "EZZ+Z". Analogously, a treatment consisting of methyl (E,Z,Z)-2,4,6-decatrienoate (4.4 mg/septum) without zingiberene was prepared (designated "EZZ"), and septa impregnated with only hexane served as controls ("C").





**Fig. 4:** Traps used for the study: A. Fabricated as previously described (ALDRICH et al. 1984) and, B. supplied by Sterling International, Inc.

Lures for a second experiment (Exp. 2) were prepared as described above for the *Thyanta*-based treatments except that a second methyl decatrienoate isomer (the Z,E,Z-isomer) was also used, and the compound previously identified as the main pheromone component of *Euschistus* spp. (ALDRICH et al. 1991), methyl (E,Z)-2,4-decadienoate (= "D"), was used instead of zingiberene. None of these lures were protected from light. Treatments for Exp. 2 consisted of EZZ, ZEZ+D, D, ZEZ, EZZ+D, and C; with four replicates per treatment.

On 10 September 2004 three additional treatments (four replicates each) were added to Exp. 2 to test the possibility of an interaction between methyl (Z,E,Z)-2,4,6-decatrienoate and the male-specific compounds previously identified from the green stink bug, *A. hilare* (ALDRICH et al. 1989; MCBRIEN et al. 2001): (Z)- $\alpha$ -bisabolene, (4S)-cis- and (4S)-trans-(Z)- $\alpha$ -bisabolene epoxides. The commercial sample of bisabo-

lene was determined to contain ~20% (Z)- $\alpha$ -bisabolene; therefore, septa were loaded with 20 mg of bisabolene so as to contain 4 mg of the desired isomer for one of the additional treatments (= "B"), with a second treatment consisting of ZEZ (4 mg) + B (10 mg). The third additional treatment consisted of a 1:1 mixture of (4S)-cis- and (4S)-trans-(Z)- $\alpha$ -bisabolene epoxides (= "X"). The amount of epoxides available was limiting (~12 mg); therefore, only one treatment included the epoxides: ZEZ (4 mg) + X (3 mg).

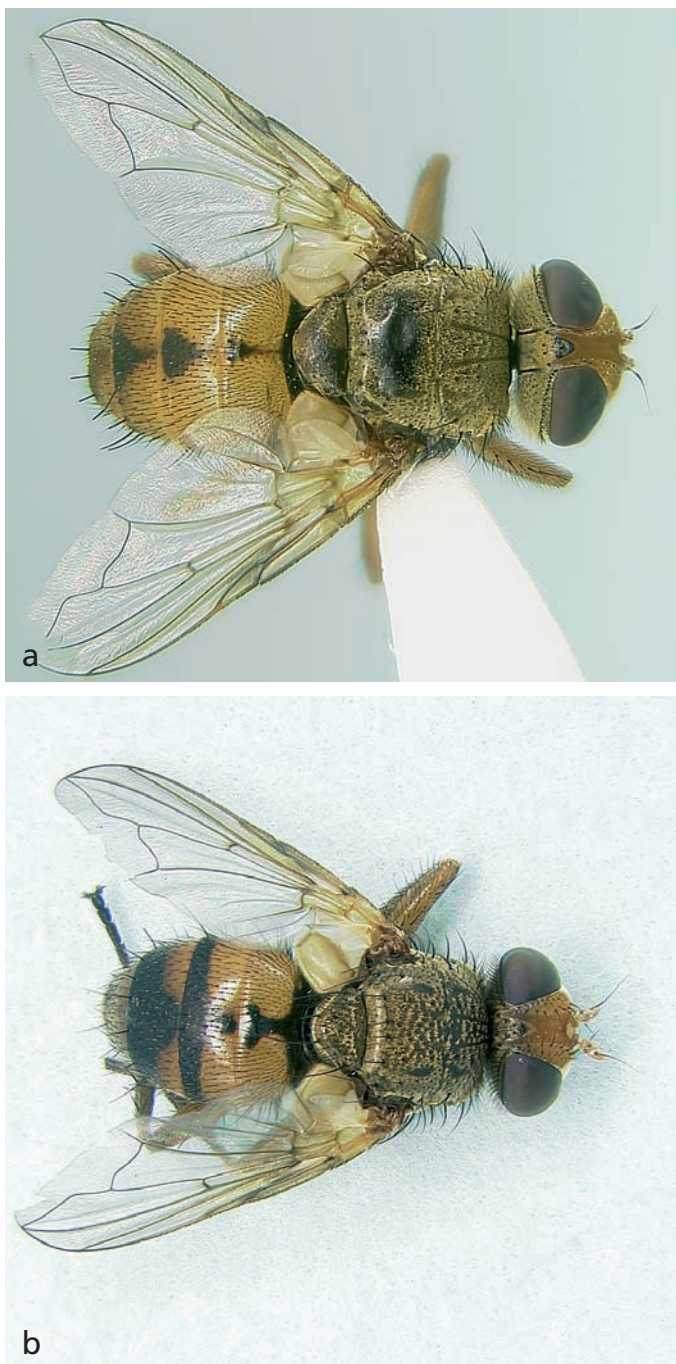
A third experiment (Exp. 3) was conducted from 9 July through 15 October 2004 using Jackson delta traps with removable sticky inserts (Agrisense, Fresno, CA) (ZHANG & ALDRICH 2004). Rubber septa (four replicates per treatment) were impregnated with chemicals as described above except that each septum was loaded with 2-mg active ingredient/lure, lures were pinned inside PVC pipe as in Exp. 1, and hung from

**Table 1:** Chemical treatments tested.

<sup>a</sup> Polyvinyl chloride. <sup>b</sup> Custom made clear plastic funnel trap (Figure 4A). <sup>c</sup> Lure pinned inside PVC pipe to shield against sunlight. <sup>d</sup> Clear plastic funnel trap constructed and supplied by Sterling International, Inc. (Figure 4B). <sup>e</sup> Lure shielded from direct sunlight by pinning inside trap roof. <sup>f</sup> Jackson delta traps with removable sticky inserts (Agrisense, Fresno, CA).

Exp.	Code	Compound(s)	Formulation	Trap
1	E-2H+ $\alpha$ -T	(E)-2-hexenal + $\alpha$ -terpineol PVC <sup>a</sup> Aldrich <sup>b</sup>	1 EZZ methyl (E,Z,Z)-2,4,6-decatrienoate	septum/protected <sup>c</sup> Aldrich
1	EZZ+Z	methyl (E,Z,Z)-2,4,6-decatrienoate + zingiberene	septum/protected	Aldrich
2	U-EZZ	methyl (E,Z,Z)-2,4,6-decatrienoate	septum/unprotected	Sterling <sup>d</sup>
2	U-D	methyl (E,Z)-2,4-decatrienoate	septum/unprotected	Sterling
2	U-EZZ+D	methyl (E,Z,Z)-2,4,6-decatrienoate + methyl (E,Z)-2,4-decatrienoate	septum/unprotected	Sterling
2	U-ZEZ	methyl (Z,E,Z)-2,4,6-decatrienoate	septum/unprotected	Sterling
2	U-ZEZ+D	methyl (Z,E,Z)-2,4,6-decatrienoate + methyl (E,Z)-2,4-decatrienoate	septum/unprotected	Sterling
2	U-ZEZ+B	methyl (Z,E,Z)-2,4,6-decatrienoate + $\alpha$ -bisabolene	septum/unprotected	Sterling
2	U-X	bisabolene epoxide	septum/unprotected	Sterling
2	U-ZEZ+X	methyl (Z,E,Z)-2,4,6-decatrienoate + bisabolene epoxide	septum/unprotected	Sterling
3	EEZ	methyl (E,E,Z)-2,4,6-decatrienoate	septum <sup>e</sup>	sticky <sup>f</sup>
3	ZEZ	methyl (Z,E,Z)-2,4,6-decatrienoate	septum	sticky
3	EEZ+ZEZ	methyl (E,E,Z)-2,4,6-decatrienoate + methyl (Z,E,Z)-2,4,6-decatrienoate	septum	sticky

**Fig. 5:** *Euclytia flava*  
(TOWNSEND)  
(Tachinidae:  
Phasiinae): A. Male  
and, B. Female.



the roof of traps. Treatments consisted of a hexane controls (C), methyl (E,E,Z)-2,4,6-decatrienoate (EEZ), methyl (Z,E,Z)-2,4,6-decatrienoate (ZEZ), and EEZ+ZEZ (3:1 ratio). The lures and conditions for the three field trapping experiments are summarized in Table 1.

#### Field trapping and insects

Field experiments were carried out at the USDA Beltsville Agricultural Research Center (BARC), Prince George's County, Maryland, from June through October of

2004. Exp. 1 was conducted at the South Farm of BARC from 15 June through 17 September using traps made as previously described (ALDRICH et al. 1984) (Fig. 2A) that were hung about 1.8 m above ground from the branches of trees ( $\geq 20$  m apart) in a mixed deciduous forest bordering agricultural fields. Exp. 2 was conducted at the North Farm of BARC from 1 July through 26 October using traps (Fig. 2B) supplied by Sterling International, Inc. (Spokane, WA) and deployed as for Exp. 1. Traps with the H+ $\alpha$ -T treatment were rebaited every 2-3 days; traps with the other treatments on rubber septa were rebaited every two weeks. Usually traps were monitored daily; insects caught inside, on and within  $\sim 1$  m of a trap were counted. Field-collected insects were preserved for species identification, and used for further laboratory experimentation. Insect photographs were taken using the Montage System (Synchroscope, Frederick MD).

#### Tachinid oviposition tests

Colonies of *P. maculiventris* and *T. custator accerra* were started from field-collected adults and nymphs, and maintained as previously described (ALDRICH et al. 1984; ALDRICH et al. 1991). Female tachinid flies caught in pheromone-baited traps were brought into the laboratory and confined in cages with adults of 2-4 species of pentatomids (either laboratory reared or field-collected) for 24 hr as previously described (ALDRICH 1995a). After exposure of the adult stink bugs to tachinid females, the bugs were removed and examined to record the number of eggs laid on each bug.

#### Gas chromatography-electroantennogram detector (GC-EAD) analysis

A Hewlett Packard 6890 gas chromatograph equipped with a 60 m x 0.25-mm ID, 0.25- $\mu$ m film-thickness DB-WAXetr capillary column (J&W Scientific Inc., Folsom, California) in the splitless mode with nitrogen as carrier (2 ml/min) was used for GC (50 °C for 2 min, then programmed to 230 °C at 10 °C/min and held for 10 min). Antennal recordings were made using a holder constructed from a piece of acrylic plastic (8 cm long x 0.8 cm wide x 0.6 cm thick) into which two 1.25-mm diameter wells were



drilled near the end of the plastic about 3 mm apart with a notch cut between the two wells to allow airflow. Each well was filled with 0.9 % NaCl solution, and gold wires immersed in each saline-filled well electrode were connected to a high-impedance 1:100 amplifier with automatic baseline drift compensation. The EAD preparation was made using the whole head of a fly immersed in one well with the tips of the antennae making contact with the saline in the other well via a short lateral capillary tube inserted in the side of the other well. The antennal preparation was cooled to ~5 °C inside a condenser by circulating near 0 °C water from a bench top refrigeration unit (RTE-100, NESLAB instruments, Inc., Portsmouth, NH). The flame ionization and electrophysiological output signals (effluent split in 1:1 ratio) were recorded using Hewlett-Packard ChemStation™ software. Standard solutions for GC-EAD were prepared at a concentration of ~10 ng of each standard/ $\mu$ l  $\text{CH}_2\text{Cl}_2$ .

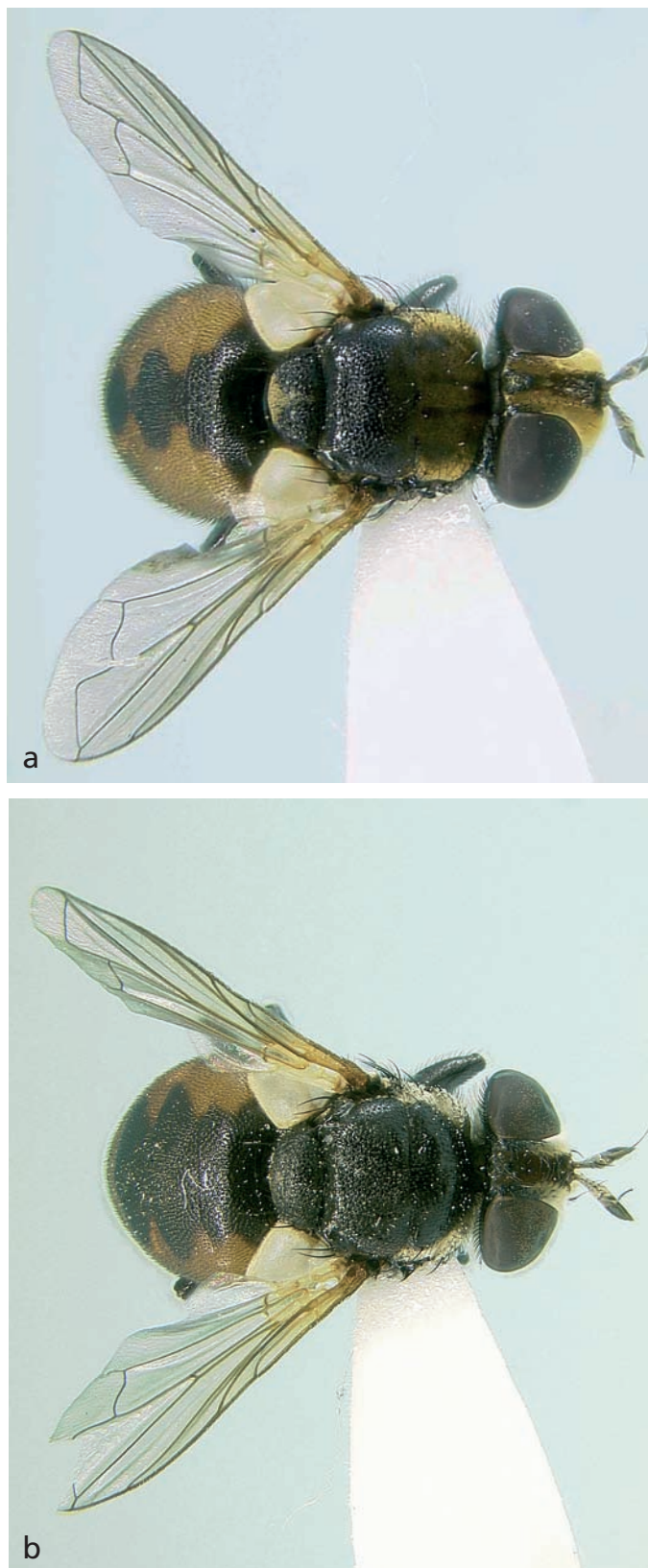
### Statistical analysis

The chi-square test was used to compare egg totals for oviposition choice tests. Data for *E. flava* captures in Exp. 1 were analyzed using ProcGenmod with chemical treatments as the factor and a Poisson error distribution. When a treatment was statistically significant, the means were compared with Sidak adjusted p-values so that the experiment-wise error was 0.05 (ANONYMOUS 2004).

## Results

### Field trapping and insects

During Exp. 1 the following known tachinid parasitoids of stink bugs were captured: *Euclytia flava* (TOWNSEND) (Phasiinae) (Fig. 5), *Gymnosoma par* (WALKER) (Phasiinae) (Fig. 6), *Euthera tentatrix* LOEW (Dexiinae) (Fig. 7A), *Hemyda aurata* ROBINEAU-DESVOIDY (Phasiinae) (Fig. 7B), and *Cylindromyia fumipennis* (BIGOT) (Phasiinae) (Fig. 9A). *Euclytia flava* (20 males and 31 females) was the only species attracted to synthetic pheromones for both *P. maculiventris* and *T. custator accerra* (Fig. 8), and significantly more *E. flava* individuals were attracted to the *P. maculiventris*



**Fig. 6:**  
*Gymnosoma par*  
(WALKER)  
(Tachinidae:  
Phasiinae): A.  
Male and, B.  
Female.

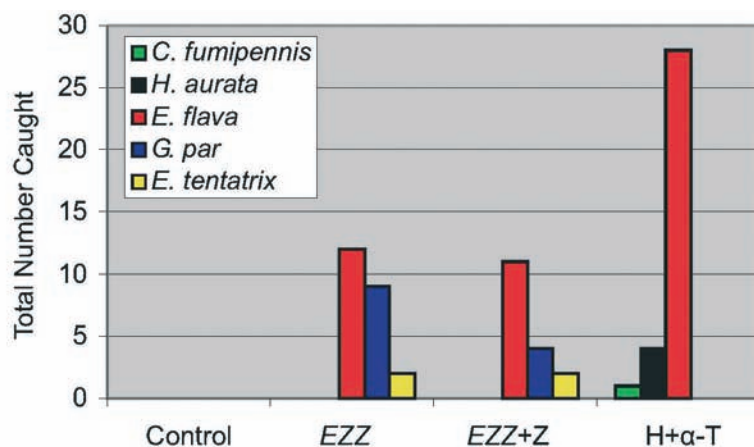
pheromone than to blends for *T. custator accerra* ( $P < 0.05$ ). The addition of zingiberene to methyl (E,Z,Z)-2,4,6-decatrionoate did not increase the attraction of *E. flava*. One female *E. flava* captured in a *T. c. accerra*

**Fig. 7:** A. *Euthera tentatrix* LOEW (Tachinidae: Dexiinae) and, B. *Hemyda aurata* ROBINEAU-DESVOIDY (Tachinidae: Phasiinae), the abdominal markings of which create the illusion of a petiole and gaster mimicking a stinging wasp.



pheromone baited trap was much smaller than the normal body-size range of individuals (ALDRICH 1986) caught in *P. maculiventris* pheromone-baited traps. Only four *H. aurata* individuals were caught in *P. maculiventris* pheromone-baited traps, but this species is known to not be effectively captured in the plastic funnel traps used for this

**Fig. 8:** Tachinids caught in traps baited with *Thyanta* versus *Podisus* pheromones, 15 June - 17 September, 2004.



test (ALDRICH et al. 1984). The single male of *C. fumipennis* caught in a *P. maculiventris* pheromone-baited trap was dissected for species determination; therefore, the specimen depicted in Fig. 9A is of an undetermined species of *Cylindromyia* (probably *fumipennis*) caught during earlier *P. maculiventris* pheromone testing. *Gymnosoma par* and *E. tentatrix* were only caught in *T. c. accerra* pheromone-baited traps and, as with *E. flava*, the inclusion of zingiberene in the lure appeared not to affect attraction of these flies (Fig. 8). No tachinids were caught in control traps.

During Exp. 2, again no tachinids were captured in control traps, while four species were caught in traps baited with pheromone treatments: *E. flava*, *G. par*, *E. tentatrix*, and *Trichopoda pennipes* (F.) (Phasiinae) (Fig. 9B). *Euclytia flava* was the most abundant tachinid species attracted (35 males and 41 females); this species was not attracted to the known *Euschistus* pheromone component (methyl (E,Z)-2,4-decadienoate), but was attracted to lures containing either methyl (E,Z,Z)-2,4,6-decatrienoate or methyl (Z,E,Z)-2,4,6-decatrienoate (Fig. 9). Conversely, *G. par* (69 individuals; sex not determined) was relatively insensitive to the methyl decatrienoate esters used in this test, but was highly attracted to lures containing methyl (E,Z)-2,4-decadienoate. *Euthera tentatrix* was weakly attracted to the methyl decadienoate and decatrienoate esters. No *T. pennipes* were caught until late in the season when a lure containing (4S)-cis- and (4S)-trans-(Z)- $\alpha$ -bisabolene epoxides was deployed (Fig. 9; 10 September - 26 October, 2004).

A total of 96 individuals of *G. par* were caught during Exp. 3, the great majority of which were female (82 females versus 14 males) (Fig. 11). Furthermore, the results of this test demonstrated that *G. par* absolutely discriminates between the two methyl decatrienoate isomers tested based on the geometry of the double bond in the 2-position. No *G. par* were attracted to traps baited with methyl (Z,E,Z)-2,4,6-decatrienoate, and about half as many flies were attracted to the combination lure. As in prior experiments, no tachinids were caught in control traps.

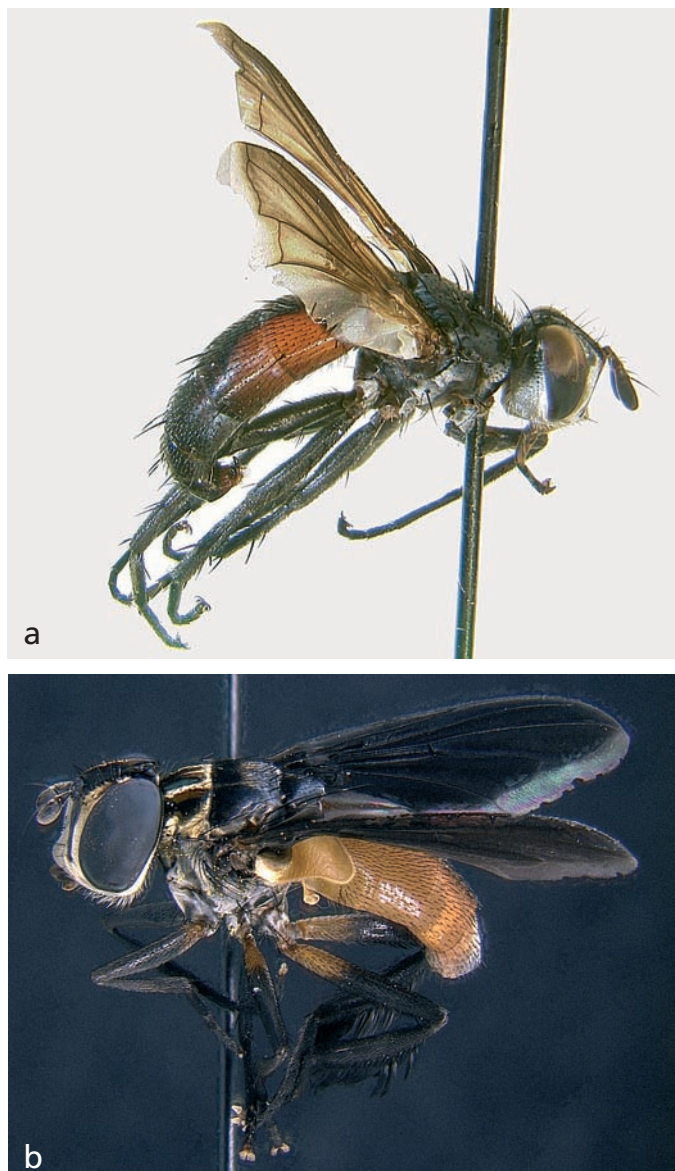


## Tachinid oviposition

Exposure of pentatomids to tachinid flies both inside pheromone-baited field traps (e.g. Fig. 12B) and laboratory cages (e.g. Fig. 1D) usually resulted in many eggs being laid on the bugs. The following tachinids were reared from bugs captured in pheromone-baited traps: *T. pennipes* from *A. hilare* (4 October, 2005), *E. tentatrix* from *E. tristigmus* (8 and 29 October, 2005), *Gymnosoma par* from *E. tristigmus* (6 September, 2005), and *Gymnoclytia occidua* (WALKER) from *E. tristigmus* (24 September, 2004).

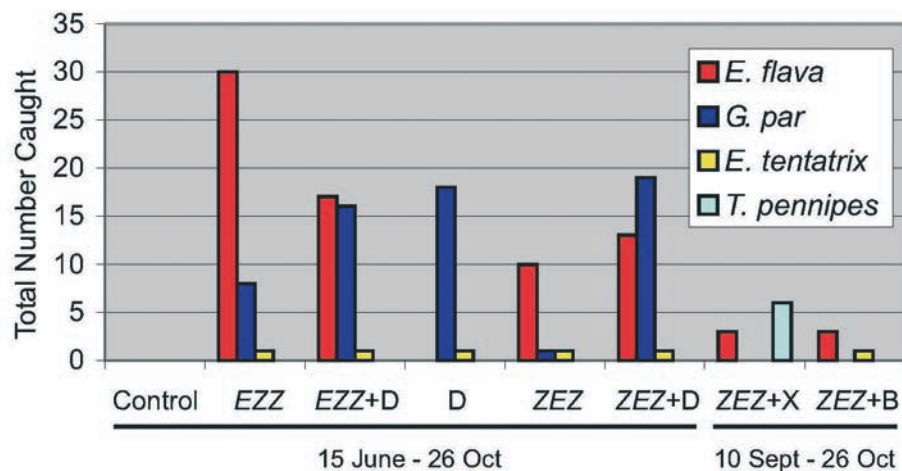
Table 2 summarizes the results of choice tests in which field-collected tachinid females were confined with adults of from 2-4 species of adult pentatomids. The total number of eggs laid on each bug was determined as a measure of the oviposition acceptability of the various pentatomids for each fly species. For *T. pennipes*, *H. halys* and *A. hilare* were clearly preferred over *E. tristigmus* and, while *H. halys* was readily accepted by *T. pennipes*, this fly still laid significantly more eggs on the known host (ARNAUD 1978), *A. hilare*. Results for *E. flava* indicated that *P. maculiventris* and *T. c. accerra* were equally acceptable for oviposition, whereas *E. tristigmus* was practically unacceptable, and *H. halys* was moderately acceptable compared to the other two species tested. Once it was realized that *G. par* females exclusively oviposit underneath the wings of potential hosts (Fig. 2B) four field-collected females were given a choice between *H. halys* and *E. tristigmus* adults; significantly more eggs were laid by *G. par* on *H. halys* versus *E. tristigmus* (Chi-square = 18.68, 1 d.f.;  $P < 0.005$ ). Finally, the single *E. tentatrix* available for oviposition testing strongly preferred *H. halys* over *E. tristigmus* when given a choice between these bugs.

Of the four tachinids commonly encountered during Exp. 1-3 (i.e. *E. flava*, *G. par*, *E. tentatrix* and *T. pennipes*), the eggs of some but not all species could be distinguished from one another. Eggs of *G. par* are larger (~2 mm) than those of the other species, somewhat oblong in shape, beige in color, and are always laid underneath the wings of bugs (Figs. 2B and 12A). Eggs of *E. flava* and *T. pennipes* are indistinguishable by

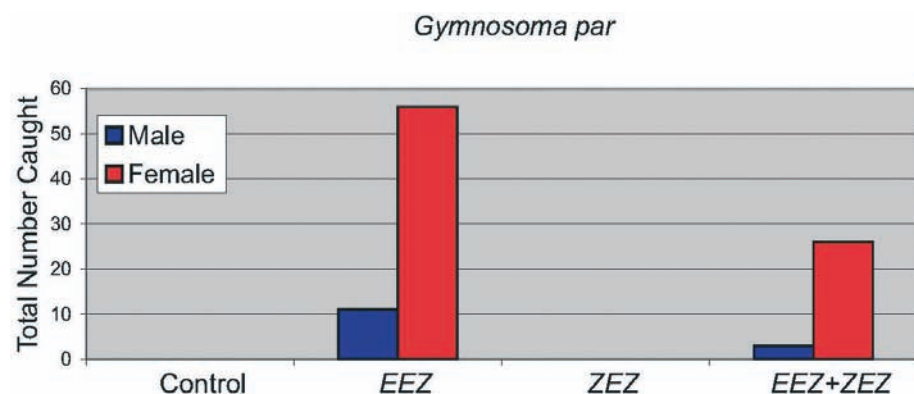


**Fig. 9:** A. *Cy lindromyia* sp. (Phasiinae) and, B. *Trichopoda pennipes* (F.) (Tachinidae: Phasiinae) showing the enlarged pulvilli characteristic of males.

light microscopy; they are ~1.5 mm long oval, and light beige to white colored. *E. flava* seem to prefer to oviposit laterally on hosts (Fig. 12B) (see also ALDRICH 1995a), whereas *T. pennipes* females seem to scatter their eggs more over the entire body of the host (Fig. 1D) (see also TODD & LEWIS 1976). Eggs of *E. tentatrix* are shaped like those of *E. flava* and *T. pennipes*, but somewhat smaller; however, they are distinctive in that they are dark brown to black in coloration (Fig. 12C), probably because species in the Dexiinae have an entirely membranous egg (STIREMAN III et al. 2006). One male *E. tristigmus* collected on 26 September, 2004, in a trap baited with methyl (E,Z)-2,4-decadienoate had a tachinid egg shaped like that of *E. flava* and *T. pennipes*, but darker beige in color than the eggs of *E.*



**Fig. 10:** Tachinids caught in traps baited with methyl 2,4,6-decatrienoates, methyl (E,Z)-2,4-decadienoate, (Z)- $\alpha$ -bisabolene epoxides, and bisabolenes (15 June - 26 October, 2004).



**Fig. 11:** *Gymnosoma par* (WALKER) caught in traps baited with methyl (E,E,Z)- and (Z,E,Z)-2,4,6-decatrienoates, 9 July - 15 October, 2004.

*flava* or *T. pennipes*. Curiously, this egg was laid precisely on one of the openings of the metathoracic scent gland (Fig. 12D).

In September and October, 2004, *H. halys* adults collected in Allentown PA were shipped alive to the Beltsville laboratory where they were examined for tachinid eggs and held for possible emergence of tachinids. Of 834 bugs examined 17 eggs were found (~2 % parasitized). A single egg was found on 6 of 374 males examined and 5 of 405 females examined, while two *H. halys* females had two eggs each and one male had two eggs. None of the eggs were laid underneath the wings of these bugs eliminating *G. par* as a possible parasitoid, nor did any fit the description of *E. tentatrix* eggs. The eggs appeared to be from either *E. flava* or *T. pennipes*. Two *T. pennipes* flies were eventually reared out of the 14 parasitized *H. halys* adults.

## GC-EAD Analysis

The antennae of *E. flava* caught late in the season in 2004 in traps baited with methyl (Z,E,Z)- or (E,Z,Z)-2,4,6-decatrienoate plus methyl (E,Z)-2,4-decadienoate were, in fact, sensitive to methyl 2,4,6-decatrienoate isomers, particularly methyl (Z,E,Z)-2,4,6-decatrienoate (e.g. Fig. 13). It should be noted that methyl (E,Z,Z)-2,4,6-decatrienoate is thermally unstable and decomposes in the GC injection port (MILLAR 1997); therefore, this compound cannot be monitored by GC-EAD. Interestingly, these antennal preparations were also highly sensitive to the main components of the pheromone of *P. maculiventris*, particularly  $\alpha$ -terpineol.

## Discussion

Tachinids in the subfamily Phasiinae commonly exploit pheromones to guide their search for potential hosts (ALDRICH 1995b). This conclusion is bolstered by the results presented here – results that we believe also provide clues to pheromones and host/parasitoid relationships yet to be discovered – but which also highlight how meager our comprehension of these relationships are. Relatively few heteropteran species have been semiochemically investigated (MCBRIEN & MILLAR 1999). Furthermore, bugs are often reluctant to actually enter traps, possibly because at close-range the substrate vibrations they normally produce to find one another are lacking in traps (ČOKL et al. 2005; VIRANT-DOBERLET et al. 2005), and tachinids are frequently territorial around traps (ALDRICH 1985) or otherwise averse to entering traps (ALDRICH et al. 1984). In the present study, initiated in part to pursue a lead that *H. halys* in Japan is attracted to methyl (E,E,Z)-2,4,6-decatrienoate isolated from *P. stali* males (TADA et al. 2001a, 2001b), the interpretation of results is somewhat complicated because these multiply unsaturated compounds tend to isomerize with time and exposure to light (KHRIMIAN 2005). Nevertheless, the synthetic routes used to produce the methyl 2,4,6-decatrienoate isomers that we tested preclude the presence of the previously known pheromone component of *Euschistus* spp. (methyl (E,Z)-2,4-decadienoate, ALDRICH et al. 1991), so we are confident



that the attraction that we observed for various species to methyl decatrienoates is not spurious.

Prior research on the pheromone system of the spined soldier bug, *P. maculiventris*, revealed that two tachinids, *E. flava* and *H. aurata*, are extremely reliant on the male-produced pheromone to find this host (ALDRICH et al. 1984). The latter species seems less abundant than the former, and in the eastern U. S. *H. aurata* also uses the pheromone of another predacious bug, *Stiretrus anchorago* (SAY), as a kairomone between generations of the spined soldier bug (Aldrich unpubl. data, KOCHANSKY et al. 1989). *Euclytia flava*, however, is seldom captured in traps baited with synthetic *P. maculiventris* pheromone in late summer or early fall, and this fly almost never emerges from overwintering spined soldier bugs caught in pheromone-baited traps in the spring, whereas about 10% of these bugs contain a maggot of *H. aurata*. Nevertheless, *E. flava* is the first tachinid to appear at *P. maculiventris* pheromone-baited traps in the spring, usually toward the end of April in Maryland (ALDRICH 1995b). Records of *E. flava* being reared from field-collected *Thyanta* spp. (OETTING & YONKE 1971; EGER & ABLES 1981; JONES et al. 1996) implied that *Thyanta* bugs might be an alternate host for *E. flava* in the second half of the growing season. Here we have shown that *E. flava* is, in fact, attracted to a known pheromone component of *T. c. accerra* (i.e. methyl (E,Z,Z)-2,4,6-decatrienoate) and that *T. c. accerra* is equally acceptable to *P. maculiventris* for ovipositing *E. flava* females (Table 2), substantiating the likelihood that this stink bug is an important host for *E. flava*. But the antennae of *E. flava* are also highly tuned to the (Z,E,Z)-isomer of methyl 2,4,6-decatrienoate (Fig. 13) and *E. flava* is attracted to this isomer (Fig. 10), suggesting that *T. c. accerra* or some other native bug may produce the (Z,E,Z)-isomer as part of its pheromone. In fact, another species of stink bug, namely *Banasa dimidiata* (SAY), was significantly attracted to an (E,Z,Z)-isomer treatment that was unprotected from light such that it could easily isomerize (ALDRICH 2005, unpublished data). Future testing of bugs in the genus *Banasa* should reveal whether or not members of this group utilize some variation of the methyl decatrienoate pheromone theme.

**Table 2:** Number of eggs laid on pentatomids simultaneously exposed to tachinid females in the laboratory.

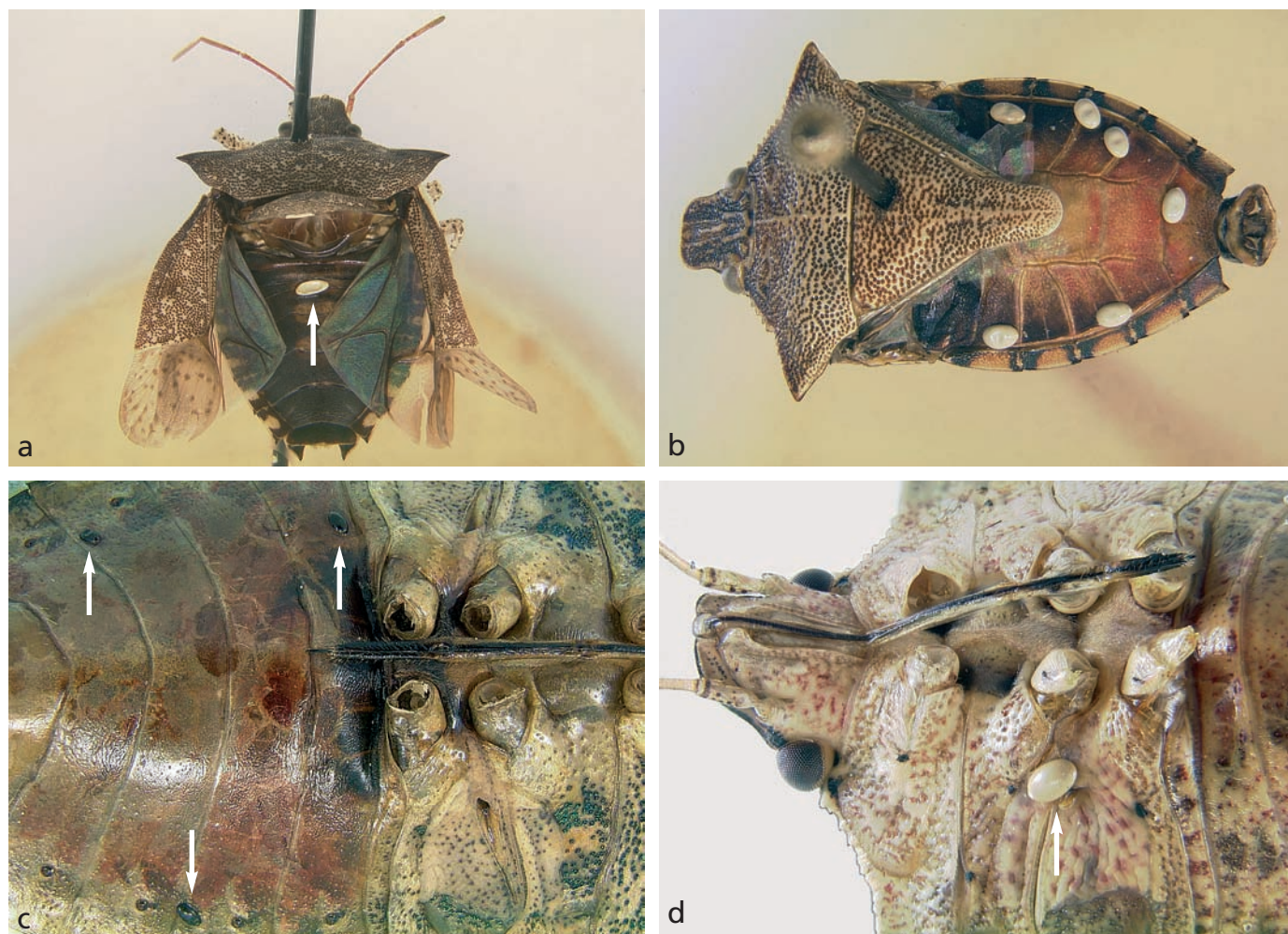
<sup>a</sup> Cages provisioned with honey, water and green beans as previously describe (ALDRICH 1995).

<sup>b</sup> Caught 20 September - 1 October in traps baited with methyl (Z,E,Z)-2,4,6-decatrienoate plus (Z)- $\alpha$ -bisabolene epoxides (see Exp. 2). <sup>c</sup> Species not included. <sup>d</sup> Caught 12-13 October in traps baited with methyl (Z,E,Z)-2,4,6-decatrienoate or methyl (E,Z,Z)-2,4,6-decatrienoate (see Exp. 2).

<sup>e</sup> Sex of pentatomid not recorded. <sup>f</sup> Caught 7 May - 30 June in traps baited with (E)-2-hexenal and  $\alpha$ -terpineol (see Exp. 1). <sup>g</sup> Caught 7-16 September in traps baited with methyl (Z,E,Z)-2,4,6-decatrienoate or methyl (E,Z,Z)-2,4,6-decatrienoate plus methyl (E,Z)-2,4-decadienoate (see Exp. 2). <sup>h</sup> Caught 5 September in trap baited with methyl (E,Z,Z)-2,4,6-decatrienoate and zingiberene (see Exp. 1).

Pentatomid Species (M = male; F = female)						
Fly Species	Fly No.	Days <sup>a</sup>	<i>H. halys</i>	<i>E. tris.</i>	<i>A. hilare</i>	<i>P. mac.</i> <i>T. c. accer.</i>
<i>Trichopoda pennipes</i> <sup>b</sup>	1	18	55(M)	12(M)	104(F)	— <sup>c</sup> —
	2	18	26(M)	0(F)	4(M)	— —
	3	18	18(M)	0(F)	30(M)	— —
	4	18	6(M)	6(M)	62(F)	— —
Subtotal			105	18	173	
	5	2	36(M)	0(F)	—	— —
	5	1	11(M)	—	26(F)	— —
	5	9	1(M)	—	6(M)	— —
	6	9	7(F)	—	38(F)	— —
Subtotal			19	—	70	— —
<i>Euclytia flava</i>	1-4 <sup>d</sup>	5	27 <sup>e</sup>	3	—	23 21
	1-2	1	—	3	—	23 24
	5 <sup>f</sup>	4	5	—	—	20 19
	6	7	5	—	—	18 24
	7	5	27	—	—	23 24
Subtotal			27	—	—	61 67
	8	7	4	—	—	16 —
	9	7	8	—	—	20 —
	10	14	27	—	—	76 —
	11	8	5	—	—	15 —
	12	9	2	—	—	1 —
	13	12	6	—	—	36 —
	14	25	28	—	—	44 —
	15	13	0	—	—	26 —
	16	7	1	—	—	13 —
	17	7	0	—	—	16 —
	18	12	0	—	—	32 —
	19	9	0	—	—	23 —
Subtotal			81	—	—	318 —
<i>Gymnosoma par</i> <sup>g</sup>	1-2	5	60(M)	26(F)	—	— —
	3	5	8(M)	0(F)	—	— —
	4	1	3(M)	2(M)	—	— —
Subtotal			71	28	—	—
<i>Euthera tentatrix</i> <sup>h</sup>	1	9	35(M)	3(F)	—	— —

The sensitivity of some tachinids to host pheromones and the precision of these kairomonal messages is amazing, in some cases exceeding that of the bugs for their own pheromones. For example, in early pheromone field tests for *Euschistus* spp., almost as many bugs were attracted to ethyl (E,Z)-2,4-decadienoate (known as the “pear ester”) as to the true pheromone component, methyl (E,Z)-2,4-decadienoate, but the tachinid parasitoids accurately discriminated between these molecules. Similarly, in the present study *G. par* absolutely discriminated between methyl (E,E,Z)-2,4,6-decatrienoate and methyl (Z,E,Z)-2,4,6-deca-



**Fig. 12:** A. Wild male *Euschistus tristigmus* (SAY) with an egg of *G. par* on the tergum, B. *Podisus maculiventris* (SAY) male from a pheromone-baited trap with eggs of *Euclytia flava* TOWNSEND, C. adult *Halyomorpha halys* (STÄL) confined with a female *Euthera tentatrix* LOEW showing the distinctive dark-colored eggs and, D. an adult male *E. tristigmus* with an egg of an unknown tachinid covering one opening of the metathoracic scent gland.

trienoate (Fig. 11). The fact that this abundant North American tachinid, which is known to parasitize various pentatomid species, is so finely tuned to a compound yet to be found from any native heteropteran species is probably an indication of our semiochemical ignorance; there must be one or more North American bugs that produce this compound as part of their attractant pheromone.

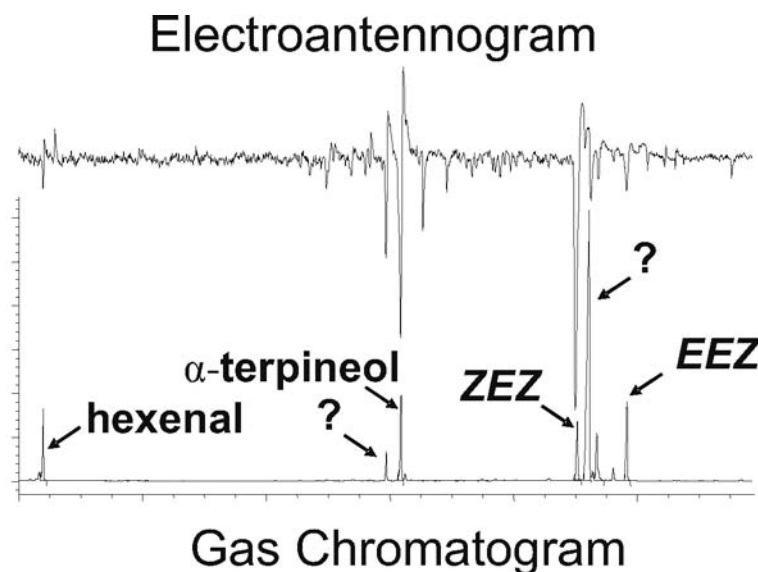
Based on the powerful attraction of *G. par* to methyl (E,E,Z)-2,4,6-decatrienoate, we expected that this parasitoid species might be pre-adapted to adopt *H. halys* as a host since circumstantial evidence suggests that this newly invasive bug might produce methyl (E,E,Z)-2,4,6-decatrienoate as part of its pheromone. Despite the preference of *G. par* females for *H. halys* over *E. tristigmus* in oviposition tests (Table 2), no eggs of *G. par* were found under the wings of the 834 adults collected in Allentown PA in 2004. Surprisingly, instead, *T. pennipes* has apparently al-

ready made the host-shift to *H. halys* in North America. Indeed, *T. pennipes* seemed unlikely to parasitize *H. halys* because in the current study this fly was only captured when presented with (Z)- $\alpha$ -bisabolene epoxides, sesquiterpenoids to date only associated with *Acrosternum* and *Nezara* spp. (ALDRICH et al. 1989), and both *G. par* and *E. tentatrix* exhibited a greater preference for *H. halys* in oviposition choice tests than did *T. pennipes* (Table 2). There are reportedly three geographically isolated strains of *T. pennipes* in the U. S. (DIETRICK & VAN DEN BOSCH 1957; PICKETT et al. 1996) evidently based, at least in part, on differential attraction to host pheromones (ALDRICH et al. 1989), but the pheromones of two of the host strains have yet to be identified. Likewise, identification of the pheromone of *H. halys* remains elusive (ALDRICH 2005, unpublished data). If and when the semiochemical gaps in our knowledge of this web of species are filled, perhaps the explanation for parasitoid host-shifts or lack thereof will become obvious. At this



point in time the situation remains mysterious. To top it all off, in the present tests the green stink bug, *A. hilare*, was significantly attracted to methyl (Z,E,Z)-2,4,6-decatrienoate but presentation of this isomer with either (Z)- $\alpha$ -bisabolene epoxides or (Z)- $\alpha$ -bisabolene did not increase attraction of this bug (ALDRICH 2005, unpublished data).

Among the phasiines host location often involves homing-in on the pheromones, but this is by no means always the case. For example, KRUPKE & BRUNNER (2003) showed that *Gymnocyttia occidentalis* TOWNSEND in the western U. S. finds *Euschistus conspersus* UHLER by going to methyl (E,Z)-2,4-decadienoate (the major male-produced pheromone component), but the other common parasitoid of the consperse stink bug, *Gymnosoma filiola* LOEW, exhibited no preference for pheromone-baited versus unbaited traps. Similarly, *Cylindromyia* spp. are commonly reared tachinids from a variety of pentatomids (Aldrich, unpublished data; OETTING & YONKE 1971; EGER & ABLES 1981; MCPHERSON et al. 1982; JONES et al. 1996), yet they are almost never seen in or around traps baited with pentatomid pheromones. One tachinid is known which lays significantly more eggs on adult females of *N. viridula* (GIANGIULIANI et al. 1991), a pentatomid whose males are thought to be the pheromone producers (ALDRICH 1988b; MCBRIEN & MILLAR 1999). As mentioned earlier, the common tachinid parasitoids of *P. maculiventris* exploit the adult male-produced pheromone as a host-finding kairomone, but these species also apparently attack nymphs by orienting to their defensive scent (ALDRICH 1988a, 1995b). In this vein, it is conceivable that the unknown tachinid which laid its egg squarely on the opening of the scent gland of *E. tristigmus* (Fig. 12D) actually was attracted and guided to this point by the bug's allomone. *Euthera tentatrix* (Dexiinae) was the only non-phasiine tachinid caught, always being found in low numbers in pheromone-treated traps (Figs. 8 and 10). Recently COLAZZA et al. (2004a, 2004b) found that feeding and oviposition by the southern green stink bug, *Nezara viridula* (L.), induces the release of host-plant volatiles attractive to the egg parasitoid,



*Trissolcus basalis* (WOLLASTON) (Hymenoptera: Scelionidae). The extent to which, if any, phasiine tachinids exhibit such tritrophic responses is not known, nor has the possibility been investigated that tachinids or other parasitoids use the substrate vibrations of heteropterans to find bugs.

Of course, finding a host is just the first step in the parasitization process of tachinids, followed by host recognition, oviposition, penetration of the tachinid larva into the host, and survival. Data presented herein and previously (ALDRICH 1995a) indicate that tachinids often oviposit on potential hosts to which they are not chemically attracted or into which they are unable to penetrate and/or survive. The cues involved in appropriate host recognition and acceptance remain essentially unknown, except that it has been shown that an extract from the cuticle of native *Euschistus* bugs applied to *N. viridula* (exotic to the U.S.) somewhat wards off oviposition attempts by native tachinids (ALDRICH 1995a). The waxy secretions produced by various pentatomoid bugs, including *Brochymena* spp. in North America (LESTON 1953), may be another chemical defense evolved to make it difficult for tachinids to stick their eggs onto the cuticle of a potential host (ALDRICH 1988b). The thick cuticle of exposed surfaces of a bug's body is also an important barrier to penetration of tachinid larvae (SHAHJAHAN & BEARDSLEY 1975). Thus, bugs are not totally defenseless against tachinid attack, and even alter their behavior so as to avoid tachinids

**Fig. 13:** Gas chromatographic-electroantennogram detector experiment using antennae from a female *Euclytia flava* caught 26 October, 2004, in a trap baited with methyl (Z,E,Z)-2,4,6-decatrienoate plus methyl (E,Z)-2,4-decadienoate to a standard mixture of isomers of methyl 2,4,6-decatrienoate and the main pheromone components of *Podisus maculiventris*, (E)-2-hexenal and  $\alpha$ -terpineol.

(ALDRICH et al. 1984) or sometimes attempt to rub off tachinid eggs (Aldrich, unpublished observations) similar to the reactions of certain parasitized caterpillars (HERREBOUT 1969). In turn, phasiines have countered these host defenses in various ways. For example, *Trichopoda plumipes* (F.) oviposits underneath the wings of *Brochymena* spp. where there is no wax protection (EGER 1981). Similarly, the habit of *G. par* (Fig. 2B) and at least one other *Gymnosoma* sp. (HIGAKI 2003) of ovipositing underneath the wings of the potential hosts (see also ALDRICH et al. 1995), as well as the habit of *Ectophasia crassipennis* (F.) to oviposit underneath the pronotum of *N. viridula* adults (COLAZZA & BIN 1990; GIANGIULIANI et al. 1991), appear to be counter-adaptations to protect eggs and facilitate larval penetration. The repeated evolution of piercing structures enabling internal oviposition by some tachinids (e.g. ALDRICH et al. 1999) is another way these flies by-pass hosts' external and behavioral defenses and, once inside a host, most tachinid larvae form a respiratory funnel derived from host defensive cells thereby circumventing encapsulation (STIREMAN III et al. 2006).

A decade ago one of us (JRA) expressed the hope that someday native natural enemies might be "taught" to recognize and accept foreign hosts as an alternative to classical biological control (ALDRICH 1995a). Ten years later this hope still remains a dream. Yet, like the proverbial wish to be a fly on the wall to overhear events unknown, studying what goes on in the brains of tachinid flies is revealing future pathways for research.

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## Zusammenfassung

Es werden Daten und Beobachtungen zur Anlockwirkung bekannter oder vermuteter Pheromone folgender Baumwanzen (Heteroptera, Pentatomidae) auf Raupenfliegen (Tachinidae, Diptera) mitgeteilt: *Podisus maculiventris* (SAY), *Euschistus tristigmus* (SAY), *Thyanta custator accerra* MCATEE, *Acrosternum hilare* (SAY) und *Halyomorpha halys* (STÅL). *Halyomorpha halys* ist eine neue, invasive Art in den östlichen U.S.A., während die anderen Arten in Nordamerika heimisch sind. Die folgenden Tachiniden wurden festgestellt: *Euclytia flava* (TOWNSEND) (Phasiinae), *Gymnosoma par* (WALKER) (Phasiinae), *Euthera tentatrix* LOEW (Dexiinae), *Hemyda aurata* ROBINEAU-DESVOIDY (Phasiinae), *Cylindromyia fumipennis* (BIGOT) (Phasiinae), and *Trichopoda pennipes* (F.) (Phasiinae). Tachiniden der Unterfamilie Phasiinae nutzen regelmäßig die Pheromone ihrer potentiellen Wirte um diese aufzuspüren. Die Befunde der vorliegenden Studie unterstützen dieses Ergebnis und liefern Anhaltspunkte für weitere Pheromone und Wirt/Parasitoid Beziehungen, die noch aufzuklären sind.

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